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Claim for review: Yes

(54) Conjugate formulated by PEG derivatives and altered interferon-alpha 2a and 2b.

Summary

This invention, an amino acid residue that does not affect the biological activity of interferon-alpha 2a or 2b, is in regards to the conjugate formulated between PEG derivatives and interferon-alpha 2a and 2b, which are altered to form a unique bond with biopolymers such as PEG derivatives, that reduces the lowering of physiological activity of interferon while increasing the residual period within the body. This conjugate formulated between the PEG derivative and interferon-alpha 2a or 2b, which has at least one of the No. 106 threonine residue or No. 161 leucine residuum metathesized as the cysteine, suppresses the reduction of the interferon's antiviral activity and increases and increases the residual period within the body. Hence, it can be very useful for treatment of hepatitis B and hepatitis C, and as an anti-cancer substance.

Specification.

Detailed explanation of the invention

Purpose of the Invention

Associative technology of the invention and the augmented technology of this field

This invention is in regards to the conjugate formulated between PEG derivatives and interferon-alpha 2a and 2b, which are altered to form a unique bond with biopolymers such as PEG derivatives, for which it reduces the lowering of physiological activity of interferon while increasing the residual period within the body.

Interferon (IFN), which has the antiviral property from its ability to prevent virus reproduction, is a glycoprotein derived from majority of cells containing nucleus that suppresses cell growth and controls immunity reactions. Interferon initiates a series of reactions within the cells by bonding with the specific acceptor of the cell membrane on the cell surface. These reactions in the cells include the derivation of specific enzyme activity and suppression of cell reproduction. As for immunity control reactions, increase in phagocytosis activity of macrophage, increase in cell toxicity of the lymphocyte for the target cells, and suppression of infected virus reproduction are included.

Currently, there are 5 known interferon in humans. There exists interferon-alpha, a secretion by peripheral white blood cells or lymph blast cells, interferon-beta, a secretion by fibroblast, interferon-gamma, a secretion by B cells, interferon-omega and interferon-tau.

Interferon-alpha is secreted when peripheral white blood cells or lymph blast cells are exposed to virus, double helix RNA, or bacterial matter and has antiviral property as well as cell reproduction resistance property by activating the natural killer (NK) cells, hence, can be used as an anti-cancer substance. Because of its antigenic and structural characteristics, 24 subtypes of interferon-alpha exist where each of the amino acid sequences share about 90% similarities between each other. Furthermore, because interferon-alpha is stable even under a strong acidic environment of pH 2, it has the advantage of lowering activity reduction that incur during the separation of recombined protein. The FDA of USA has approved Intron A, developed by Schering-Plough using recombined human interferon-alpha 2b, for treating 14 types of diseases including hairy-cell leukemia, condyloma acuminatum, Kaposi's sarcoma, and hepatitis, which indicates the clinical importance of the pleiotropic activity of interferon-alpha. [Nagabhushan, T.L. & Giaquinto, A., 1995. In Regulatory Practice for Biopharmaceutical Production].

Interferon-alpha 2 consists of 165 amino acids, and the remaining interferon-alpha protein consists of 166 amino acids. With a molar weight of 19-26 kDa, it includes a bonding of 2 disulfides between No. 1 - No.

98 and No. 29 - No. 138 amino acids.

On the other hand, PEG (polyethylene glycol) is a polymer compound with HO-(-CH_2CH_2O-)$_n$-H structure with a strong hydrophilic property and is used to increase the solubility by combining with medicine protein. The range of the molar weight of PEG bonding with the protein is approximately between 1,000 and 100,000. It is known that the toxicity is significantly lower when the PEG's molar weight is 1,000 or higher. For PEG with molar weight range of 1,000 to 6,000, they are scattered throughout the entire body and metabolized in the kidneys. And for PEG with molar weight of 40,000, they are scattered to organs, including blood and liver, and is metabolized in the liver.

The medically and pharmacologically useful proteins administered through the parenteral path are antigenic and has the disadvantage of a short bodily residual period as well as the overall poor receptivity. Frank F. Davis, in US patent No. 4,179,337, indicates that if protein and enzymes bonded to PEG are used as treatment substances, they will have the advantageous effects of PEG such as antigenic reduction, improved receptivity, and increased bodily residual period. After this patent, there are attempts being made to overcome the weakness of physiologically active protein by bonding the protein and PEG. Veronese (Veronese 11:141-152, 1985) for example is bonding ribonuclease and superoxide dismutase with PEG. In addition, Katre, in US patent No. 4,766,106 and No. 4,917,888, indicates the increase in protein receptivity by bonding the protein with polymers containing PEG, and Nitecki, in US patent No 4,902,502, has increased the bodily residual period and achieved antigenic reduction by combining PEG or polymers with recombined protein.

Aside from these advantages, there also exist defects in the PEG and protein bonds. In other words, PEG in general is combined with the protein through covalent bonding with one or more of protein's free lysine residuum, however, if the PEG bonds to the active region of the protein surface, that region is no longer able to perform the biological functions and causes the protein activity to decrease. Furthermore, the bonding between PEG and lysine residuum generally occurs randomly, hence, many types of PEG-protein compounds will exist as a conjugate. Therefore, the purification process to isolate the desired compound becomes complex and difficult. Interferon-alpha 2b for example has 8 free lysine residuum on the protein surface, and it is necessary to use a selective method to bond the PEG to the amino acid among the residuum on regions that does not affect the biological activity of the interferon.

According to US patent No. 5,382,657 by Hoffman La-Roche, if the PEG, with pyridinulozycorbonyl methyl group of 1,100 - 10,000 molar weight as its active group, is to bond non-selectively with free amine group of interferon-alpha 2a, the antiviral activity was higher when the bonded PEG's molar weight was lower. The antiviral activity for a bond with 1 PEG molecule was higher than a bond with more than 1 PEG

molecules.

Also, according to US patent No. 5,908,621 by Scherling-Plough, when a PEG of molar weight 12,000 is nonspecifically attached to the free lysine residuum of interferon-alpha 2b through carbamate bonding, administering this once a week (0.5, 10., 1.5 μg/kg) had the same effect as administering recombined interferon three times a week (3 Million International Unit) but with reduced side effects.

According to US patent No. 5,981,709 by Enzon, when succinimidyl carbonate-PEG (SC_PEG) is bonded with interferon-alpha 2b, the primary resultants were a conjugate of 1 or 2 PEGH bonds, and for cytopathic effect assay (CPE assay), a conjugate containing 2 PEG bonds displayed 7.7% antiviral activity whereas a conjugate containing 1 PEG bonds displayed 29% antiviral activity, hence, there is a decrease in protein activity reduction when 1 PEG is bonded with the protein.

For US patent No. 5,985,263 by Enzon, SC-PEG of molar weight 12,000 bonds site specifically with interferon-alpha 2b. In other words, on the surface of interferon-alpha 2b, there exist 8 free amine groups that can bond with SC-PEG. The remaining 6 after excluding Cysl and His34, which are N-terminals of interferon, are Lys residuum, and since these 3 types of amino acids each have different pKa values, site specific bonding is possible. According to an example here, the PEG selectively bonds with His34, a histidine residuum of interferon-alpha 2b, by setting the pH of the bonding reaction to around 6.0, which is the pKa value of the histidine amino acid, and as a result, among the conjugates with SC-PEG bonded to single interferon, 55% of them have selectively bonded with histidine, 20% with cysl, and 12% with lys residuum. And when measuring the antiviral activity of these conjugates, the His34 bonded conjugate displayed 50.6% activity, Lys134 conjugate displayed 38.4%, Lys31 conjugate 11.2%, Cysl conjugate 12.8%, and Lysl21 and Lysl31 conjugates displayed 27.6% activity. In a different example, benzotriazole carbonate (BTC-PEG) was bonded with interferon-alpha 2b where the molar weight of the BTC-PEG used were 5000, 12000, and 20,000.

According to US patent No. 5,985,265 by Amgen, the methoxyPEG-aldehyde of molar weight 12,000 bonds with the N-terminals of consensus interferon-alpha. The reaction has yielded a single PEG bond with the N-terminal and the conjugate's antiviral activity was 20%.

In US patent No. 6,042,822 by Enzon, the SC-PEG was bonded selectively with interferon-alpha 2b, however, the reaction pH was adjusted to 5.5-6.5 such that the SC-PEG would primary bond with His34 residuum. Furthermore, SC-PEG was primary made to bond with Lys residuum in reaction under pH 8.0-10.0. Also, sodium dodecyl sulphate (SDS) is added to the native interferon bonding reaction. If 0.1%

(w/v) of SDS is added, for an interferon conjugate of a 1 SC-PEG bond where SC-PEG has molar weight of 5,000, the residual period is 6.8 hours and the antiviral activity is 30%, and for an interferon conjugate of 2 SC-PEG bond where SC-PEG has a molar weight of 5,000, the residual period is 5.8 hours and the antiviral activity is 69%, which is an increase of more than two times. On the other hand, though the activity for native interferon was 100%, there were side effects and the residual period was only 0.17 hours.

Hoffman La-Roche uses Shearwater's PEG technology and is currently testing the bonded substance between Lys residuum of interferon-alpha 2a and branched methozyPEG, 40kDa. A dose of 180 μg is administered once a week as a hepatitis C treatment under the product name Pegasys and it is currently in phase 3 of clinical trials. Other than as an exclusive treatment, it is also in clinical trial as part of a multiple treatment. By using ribavirin or histamine dihydrochloride, the patient can increase the sustained virologic response against the virus.

Furthermore, Schering-Plough uses Enzon's PEG technology to bond a linear PEG of molar weight 12,000 to Lys residuum of interferon-alpha 2b or His residuum, and it is currently in the third phase of the clinical trial. The product name is PEG-ENTRON and it is being applied with ribavirin as multiple treatment against hepatitis C, malignant melanoma, chronic myelogenous leukemia, etc.

Interferon, which is currently on sale after receiving approval by the US FDA as a treatment for hepatitis B and C viruses, was only available as a recombined form. However, Schering-Plough received approval from the US FDA for PEG-ENTRON as a chronic hepatitis C treatment on January 2001. The PEG-ENTRON has already received approval by the European Union on May, 2000.

To summarize the contents so far, if the biopolymers such as PEG bonds with recombined protein, the protein's antigenic property decreases and the bodily residual period increases, however, causes the issue of biological activity reduction. Therefore, it would be optimal if the bodily residual period could be increased while minimizing the decrease in activity by bonding biopolymers such as PEG derivatives to recombined protein such as interferon-alpha 2a or 2b.

The technological task of what the invention trying to achieve

This invention is based on the consideration of the research results of the bonds between interferon and PEG. The purpose is to create the interferon-alpha 2a or 2b which minimizes the decrease in biological activity while it increases the bodily residual period by metathesizing the specific residuum of wild type recombined interferon-alpha 2a or 2b and bonding site selectively with PEG, and to provide such conjugate of the PEG derivative and interferon-alpha 2a or 2b.

Configuration and operation of the invention

To achieve the above purpose, we feature the metathesizing of the amino acid residuum as cysteine which has no affect on the biological activity of interferon-alpha 2a or 2b.

Here, is it ideal to have at least one of No. 106 threonine residuum and No. 161 leucine residuum, which are amino acid residuum that does not affect the biological activities.

Also, the invention provides interferon-alpha 2a or 2b and conjugate of PEG derivative as above. Here, the molar weight of 5,000-100,000 is ideal for PEG derivative, and they can be linear methoxyPEG-maleimid, branched PEG-maleimide, or pendant PEG-maleimide. Furthermore, in regards to these bonds, the PEG derivative's mole ratio per mole of interferon-alpha 2a or 2b should ideally be in the 1-10 times range.

Unlike the traditional technology of Hoffman La-Roche and Schering-Plough that uses wild type recombined interferon-alpha 2a or 2b, this invention creates a site-directed mutagenesis on the amino acid residuum, which does not affect the biological activity of interferon-alpha 2a or 2b, and bonds the PEG derivative on that region using a site selective method. Therefore, the invention was able to minimize the reduction of interferon's biological activity while increasing the bodily residual period by several times. In other words, by metathesizing the No. 106 or 161 amino acid residuum as cysteine during the cloning process of recombined interferon-alpha 2a and 2b protein, and by selectively bonding linear PEG, branched PEG, or pendant PEG, which all have the ability to selectively bond with sulfhydryl of the metathesized cysteine residuum, it reduced the occurrence of immune reaction, minimized the decrease in interferon's activity, increased the bodily residual period, and achieved the same effect as the conventional wild or recombined interferon-alpha 2 protein while reducing the administered dosage and frequency.

This invention is explained in further detail below.

Interferon-alpha 2a and 2b consists of 165 amino acids where 164 of amino acid sequence are identical with the only difference being No. 23 amino acid residuum. In other words, No. 23 residuum is lysine for interferon-alpha 2a and arginine for 2b.

Sequence numbers 1 and 2 represent a base sequence and amino acid sequence of wild human interferon-alpha 2a DNA, and sequence numbers 3 and 4 represent a base sequence and amino acid sequence of the wild human interferon-alpha 2b DNA.

In this invention, the reason for selecting No. 106 threonine residuum and No. 161 leucine residuum of the interferon-alpha 2a and 2b as the site-directed mutagenesis region are as follows.

The 4 flexible areas of interferon-alpha 2, in other words, 5 residuum (1-5) of N-terminal, 6 residuum of AB3 loop (45-50), 9 residuum of CD loop (103-111), and 6 residuum of C-terminal (160-165) are areas that are exposed to the solvent. Hence, they are thought of as having ease of bonding with PEG molecule. Given a 3 dimensional structure, it exists leaning against one end. Also, when considering the report that the deletion of 4 residuum of N-terminal (1-4), 10 residuum of CD loop (102-111), and 10 residuum of C-terminal (156-165) does not affect the antiviral activity of interferon-alpha 2 [Valenzuela 1985, 313:698-700: Edge 1986, 7:1-46, Levy 1981, 78:6186-6190], it can be predicted that metathesizing No. 106 and 161 residuum as cysteine would not affect the interferon-alpha 2 activity.

(1) Reason for selecting 106 residuum for site-directed mutagenesis

Ramaswamy (Ramaswamy 1996.4: 1453-1463), using a 3-dimensional structure, revealed that the 12 amino acid region between the No. 100 isoleucine residuum and 113 glutamate residuum of interferon-alpha 2 is considerably mobile, in other words, it is the region that is exposed to the external environments.

Also, Sternberg (Sternberg 1982,4:137-144) revealed that the deletion of 10 residuum ranging from 101 to 110 of the amino acid residuum, referred to as CD loop of the interferon-alpha 2, had no effect on the interferon's activity.

Klaus (Klaus 1997,274:661-675) reported that the CD loop is located on the direct opposite side of the region that is suspected of bonding with the acceptor of interferon-alpha 2.

Furthermore, it is known that CD loop does not bond with other regions on the interferon-alpha 2.

(2) Reason for selecting 161 residuum for site-directed mutagenesis

Weczel (Weczel 1982,pp. 365-376) revealed that the C-terminal 15 amino acids of interferon-alpha 2, which includes No. 161 residuum, can be bonded with PEG since it does not have any vital functions in regards to the biological activity of the interferon.

Furthermore, Edge (Edge 1986, 7: 1-46) reported that in the wild interferon-alpha 2, antivirus, reproductive suppression, and natural killer cell activity, there were no differences between the interferon alpha 2(1-160)

with C-terminal 5 amino acids missing and interferon-alpha 2(1-155) with 10 amino acids missing.

Arnheiter (Arnheiter 1983, 80: 2539-2543) revealed that the No. 150-165 residuum region for interferon-alpha 2 is the epitope region where the antibody with high molar weight bonds with strong chemical attraction, and such antibody mildly prevents the bonding between this acceptor and the interferon-alpha 2, hence, causing no effect on the interferon activity, and that as a result, the optimal region to bring forth the cysteine residuum to bond with PEG is the 157-165 residuum.

In consideration of the above research results, this invention used at least one of No. 106 threonine and No. 161 leucine amino acid from interferon-alpha 2a and 2b to metathesize as cysteine from the DNA base sequence to produce a site-directed mutagenesis. Such fabrication of mutants can be implemented effectively through existing molecular biological technologies and methods. For example, Valenzuela (Valenzuela 1980, 134: 1404-1411) illustrates the process of deriving site-directed mutagenesis to the interferon-alpha 2.

Sequence numbers 5 and 6, in accordance with actual examples from this invention, indicate the base sequence and amino acid sequence of the human interferon-alpha 2a DNA that has derived a site-directed mutagenesis to cysteine from No. 106 threonine and No. 161 leucine residuum. Sequence numbers 7 and 8, in accordance with actual examples from this invention, indicate the base sequence and amino acid sequence of the human interferon-alpha 2b DNA that has derived a site-directed mutagenesis to cysteine from No. 106 threonine and No. 161 leucine residuum.

This invention provides a bonded conjugate by bonding thioether with PEG and human interferon-alpha 2a or interferon-alpha 2b which its No. 106 threonine residuum and/or No.161 leucine residuum has been metathesized as cysteine. In other words, the conjugate specified in this invention is formed by selectively bonding special chemical reactors that can bond with sulfhydryl group if the metathesized cysteine residuum, such as linear PEG containing maleimide, branched PEG, or pendant PEG.

Here, linear Peg can be, for example, methoxy PEG maleimide, and for molecular type PEG, it is ideal to have a bonded structure in the branched form of 2 PEG molecules with glycine as its backbone. For pendant PEG, it is ideal to have a fixed interval bonded structure in a small arm form of responders that includes 2 to 20 maleimide group with PEG molecules, with molar weight ranging between 10,000 to 40,000 Da, as its backbone.

A unique bond between these PEGs and human interferon-alpha 2a or 2b is achieved by reacting the PEG, which has the reactive group bonded that includes S-pyridyl group or maleimide of interferon-alpha 2a or

2b derivative derived as a mutant intended to carry cysteine residuum in accordance with the invention, for several to 24 hours under pH 6-7.5 conditions.

Through the example below, there are more detailed explanations of the invention. However, these examples are merely exemplifications, and the invention is not restricted to just these examples.

[Example 1]

(1)

Following the methods of Maniatix (Maniatix T. Cold Spring Harbor Laboratory, 1982), through polymerase chain reaction, the wild human interferon-alpha 2a and interferon-alpha 2b were cloned from the human complimentary DNA library. The exact size of cDNA was verified as 1.1 kb through electrophoresis. The nucleotide sequence was verified through Sanger's dideoxy sequencing.

Sequence numbers 1 and 2 represent a base sequence and amino acid sequence of wild human interferon-alpha 2a DNA, and sequence numbers 3 and 4 represent a base sequence and amino acid sequence of the wild human interferon-alpha 2b DNA.

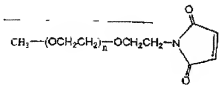
The No. 106 threonine and/or No. 161 leucine residuum of interferon-alpha 2a and 2b were metathesized as cysteine in its DNA base group sequence state through site-directed mutagenesis. Such fabrication of mutants can be implemented effectively through existing molecular biological technologies and methods (Valenzuela 1986, 134: 1404-1411). Sequence numbers 5 and 6, in accordance with actual examples from this invention, indicate the base sequence and amino acid sequence of the human interferon-alpha 2a DNA that has derived a site-directed mutagenesis to cysteine from No. 106 threonine and No. 161 leucine residuum. Sequence numbers 7 and 8, in accordance with actual examples from this invention, indicate the base sequence and amino acid sequence of the human interferon-alpha 2b DNA that has derived a site-directed mutagenesis to cysteine from No. 106 threonine and No. 161 leucine residuum.

[Example 2]

Dried diethyl ether that does not contain peroxide was added to the methoxy PEG-maleimide of molar weight 5,000, precipitated, and then, ether cleansed. The product was vacuum dried. Then, the purity and range of molar weight was measured through nuclear magnetic resonance method and gel permeation chromatography to verify whether it would be adequate for bonding with PEG. In this example, the utilized PEG was produced by SunBio Corporation.

The following structural formula indicates the methoxy PEG-maleimide of molar weight 5,000 where n is an integer from 110 to 115.

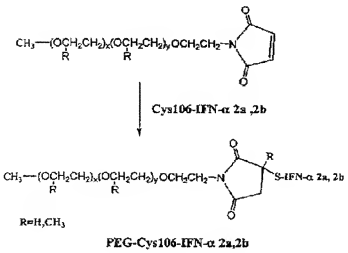
Chemical Formula 1



In accordance with example 1, the verified product was bonded with mutant derived interferon-alpha 2a or 2b under pH 6-7.5 sodium phosphate (NaH_2PO_4) buffer conditions. For buffer, DTT (dithiothreitol), EDTA (ethylenediaminetetraacetate), and guanidine hydrochloride (guanidine HCL) were included. The bonding reaction was carried out for 2 to 24 hours under 4°C. The bond resultant was analyzed by electrophoresis using SDS acrylamide gel.

The following chemical equation indicates the bonding method between the mutant derived interferon-alpha 2a or 2b and linear PEG-maleimide. Here, x+y=n where n is an integer from 110 to 115.

Chemical Formula 1



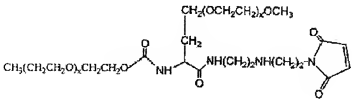
PEG-Cys106-IFN-α 2a,2b

[Example 3]

Dried diethyl ether that does not contain peroxide was added to the branched PEG of molar weight 40,000, precipitated, and then, ether cleansed. The product was vacuum dried. Then, the purity and range of molar weight was measured through nuclear magnetic resonance method and gel permeation chromatography to verify whether it would be adequate for bonding with PEG.

The following structural formula indicates the branched polyethyleneglycol of molar weight 40,000 where x is an integer from 45 to 460.

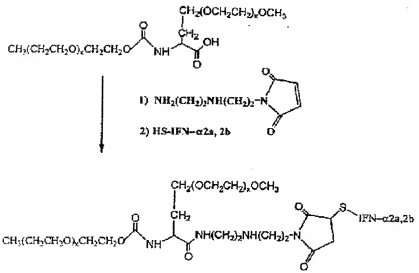
Chemical Formula 2



In accordance with example 1, the verified product was bonded with mutant derived interferon-alpha 2a or 2b under pH 6-7.5 sodium phosphate (NaH_2PO_4) buffer conditions. The conditions for bond reaction were set to be identical to example 2.

The following chemical equation indicates the bonding method between the mutant derived interferon-alpha 2a or 2b and branched PEG. Here, x is an integer from 450 to 460.

Chemical Formula 2



[Example 4]

(1)

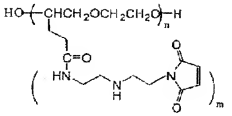
When reacting with PEG of molar weight range of 10,000 to 40,000 Da with acrylic acid, a PEG-propionic acid, a bond in an arm-like pendant form between propionic acid and PEG backbone, was fabricated by reacting with t-butyl peroxybenzoate as the initiator and nonane as dispersing agent. The pendant number (m value from Chemical Equation 3) can be adjusted in the range of 2 to 20 by regulating the equivalent weight of the acrylic acid being added.

Through the reaction of the fabricated PEG-proprionic acid diethylenetriaminylmaleimide, N,N'-dicyclohexyl carbodimide, and 4-dimethylamino-pyradine, the pendant PEG-maleimide was fabricated.

It can also be fabricated by first causing a reaction between the fabricated PEG-proprionic acid with N-hydroxy succinimide and N.N'-dicychlohexyl carbodimide, and then causing a reaction with diethylenetriaminylmaleimide.

The following structural formula indicates the pendant polyethyleneglycol of molar weight 10,000 to 40,000 where n is an integer from 110 to 460 and m is an integer from 2 to 20.

Chemical Formula 3



(2)

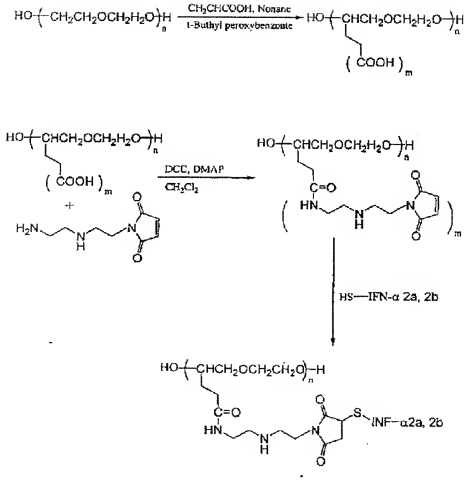
Dried diethyl ether that does not contain peroxide was added to the pendant PEG-maleimide of molar weight 10,000 to 40,000, precipitated, and then, ether cleansed. The product was vacuum dried. Then, the purity and range of molar weight was measured through nuclear magnetic resonance method and gel permeation chromatography to verify whether it would be adequate for bonding with PEG.

In accordance with example 1, the verified product was bonded with mutant derived interferon-alpha 2a or 2b under pH 6-7.5 sodium phosphate (NaH_2PO_4) buffer conditions. The conditions for bond reaction were set to be identical to example 2.

The following chemical equation indicates the fabrication of the pendant PEG and the bonding between pendant PEG and mutant derived interferon-alpha 2a or 2b where n is an integer from 110 to 460 and m is an integer from 2 to 20.

Here, the mutant derived interferon 2a and 2b molecules, derived from 1 molar weight of pendant PEG-maleimide, can bone within the range of 2 to 10.

Chemical Formula 3



[Effects Example]

Based on example 3, the conjugate formed from bonding interferon-alpha 2a and branched PEG-maleimide of molar weight 40,000 was subjected to measurement of bodily residual period and antiviral activity, and comparison of its results against interferon-alpha 2a.

The results are displayed on the following table.

[Table 1]

Interferon	Bodily residual period (t1/2)	Antiviral activity (% In vivo activity)
Recombined interferon-alpha 2a	3-5 hours	100%
Branched PEG-interferon-alpha	50-80 hours	90-100%

2a		

As shown on the above table, based on the invention, after metathesis of No. 106 threonine and/or No. 161 leucine residuum as cysteine at the recombined interferon-alpha 2a or 2b, the level of antiviral activity identical to the conjugate formed from the PEG-derivative bond is maintained while the bodily residual period becomes greatly increased.

The effect of the Invention

Based on the invention, as reviewed above, as a biopolymer that can uniquely bond with the cysteine residuum at the altered interferon-alpha 2a or 2b, which had metathesis of No. 106 threonine and/or No. 161 leucine residuum as cysteine through the site-directed mutagenesis of wild human interferon-alpha 2a or 2b, the conjugate formed between PEG-derivative bonded interferon-alpha 2a or 2b and PEG can suppress the reduction of interferon's antiviral activities and increase the bodily residual period, which allows its effective use as treatment for hepatitis B or hepatitis C, and as an anti-cancer substance.

(57) Scope of claim

Claim 1. A human interferon-alpha 2a or 2b for which its amino acid residuum, which does not affect the biological activity of human interferon-alpha 2a or 2b, has been metathesized as cysteine.

Claim 2. In reference to Claim 1, an interferon-alpha 2a or 2b for which its amino acid residuum is either one of No. 106 threonine residuum or No. 161 leucine residuum.

Claim 3. A polynucleotide that codes the interferon-alpha 2a or 2b for which its amino acid residuum, which does not affect the biological activity of human interferon-alpha 2a or 2b, has been metathesized as cysteine.

Claim 4. In reference to Claim 3, polynucleotide for which its amino acid residuum is either one of No. 106 threonine residuum or No. 161 leucine residuum.

Claim 5. In reference to Claim 3, a polynucleotide that is notated by sequence number 5.

Claim 6. In reference to Claim 3, a polynucleotide that is notated by sequence number 7.

Claim 7. A conjugate formed by the bond between Claim 1's human interferon-alpha 2a or 2b

and PEG-derivative.

Claim 8. In reference to Claim 7, a conjugate for which its PEG derivative is at least one of linear methoxy PEG-maleimide, branched PEG-maleimide, or pendant PEG-maleimide.

Claim 9. In reference to Claim 7, a conjugate for which its PEG-derivative is of molar weight between 5,000 to 100,000.

Claim 10. In reference to Claim 7, a conjugate formed by the bond between 1 to 10 moles of PEG-derivative with 1 mole of interferon-alpha 2a or 2b.

Certification

I, Chuck Kahng, testify that the English translation is a true and accurate translation of the Korean Patent (2002-0067105) attached.

Signature: Date: 1/29/2004